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2.	Patent application number *(The Patent Office will fill in this part)* 9909469.0	23 APR 1998
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	JOHN A. WILKINSON C/O SCHOOL OF HEALTH, BIOLOGICAL AND ENVIRONMENTAL SCIENCES MIDDLESEX UNIVERSITY ENFIELD EN3 4SF
	Patents ADP number *(if you know it)*	7647890001
	If the applicant is a corporate body, give the country/state of its incorporation	UNITED KINGDOM
4.	Title of the invention	INSECTICIDAL COMPOSITION
5.	Name of your agent *(if you have one)* "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	BOULT WADE TENNANT ~~27 FURNIVAL STREET~~ ~~LONDON~~ ~~EC4A 1PQ~~ Verulam Gardens 70 Grays Inn Rd. London WC1X 8BT
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11 I/We request the grant of a patent on the basis of this application.

Signature: Boult Wade Tennant

Date: 23 April 1999

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INSECTICIDAL COMPOSITION

The present invention relates to plant essential oils and extracts and their uses as insecticides both in medical and non-medical applications. In particular the invention relates to the oils and extracts of Spanish sage (*Salvia lavandulifolia;* syn. *S. lavandulaefolia*) and *S. officinalis* "petite feuille Banon" and other essential oils or plant extracts containing relatively high concentrations of sabinene, such as those from *Artemisia dracunculus* (Tarragon), *Citrus limon* (Lemon), *Juniperus communis* (Juniper), *Laurus nobilis* (Bay), *Myristica fragrans* (Nutmeg), *Origanum vulgare* (Oregano), *Piper cubeba* (Cubebs), *Aloysia gratissima* (Whitebrush) and species of *Salvia* other than *S. lavandulifolia*.

Sucking lice (*Anuplura*) are a common pest of humans and animals which have a very wide global distribution, and are often spread by physical contact. In humans, lice are of two main genera. Lice of the genus *Pediculus* include head lice (*Pediculus humanus capitis* syn. *P. capitis*) and clothing lice (*P. humanus humanus* syn. *P. corporis*), whilst the commonest lice of the genus *Pthirius* are the crab (or public) lice, *Pthirius pubis*.

Head lice are a very common problem in children, from whom the insects can then be spread to other family members. Infestations are by no means limited solely to children. In the strictest sense, lice are obligate parasites, and hence are unlikely to leave a host voluntarily, yet whether infestations can be spread by inanimate objects, such as combs or hairbrushes remains a controversial issue. Louse-infestations are usually accompanied by itching,

although it is also possible that the insects could act as vectors for bacteria responsible for skin complaints, such as impetigo and scalp pyoderma (see Burgess (1995) *Advances in Parasitology* 36:271-342). In the UK, infestation rates were recorded as high as 23.1 % in primary school-aged children, and higher still (up to 30.3 %) in secondary schools. Other surveys suggest that one third of primary/junior school children are infested at least once per year (see Gratz (1997) *Human Lice- their prevalence, control and resistance to insecticides*, WHO).

There is evidence that a recrudescence of clothing lice, which are generally rarer than head lice in "developed" countries, is occurring (see Gratz (1997) *Human Lice- their prevalence, control and resistance to insecticides*, WHO). These insects have been linked to the transmission of trench fever, relapsing fever and typhus (see Van Der Lann and Smit (1996) *Nederlands Tijdschrift voor Geneeskunde* 140: 1912-1915).

Conventional treatment of head lice depends on application of insecticidal compositions and/or physical removal of lice and nits (lice eggs) by mechanical means (usually a fine-toothed comb). Insecticides used for treatment of head lice include organophosphates (e.g. malathion), pyrethroids, lindane and DDT. There are a number of problems associated with these compounds, including health concerns over exposure to organophosphates, development of resistance by lice (see Gratz (1997) *Human Lice- their prevalence, control and resistance to insecticides*, WHO) and an undesirable product formulation (e.g. unpleasant smell). Some preparations also contain very high (>80% v/v) concentrations of alcohol, which has been linked to

instances of allergenic reactions.

A number of plant-derived treatments for lice have been developed previously, ranging from *Stemosa tuberosa* and *Hyssop officinalis* extracts, quassia chips, pyrethrins, rotenone (from *Derris* or *Lonchocarpus* spp.) to a number of essential oils (including Eucalyptus and Rosemary). A number of these preparations have, however, been found to be of limited efficacy or have been associated with mammalian toxicity (see Burgess (1995). *Advances in Parasitology* 36:271-342). US patents 5,227,163 and 5,411,992 to Eini claim that a number of essential oils, including sage, and also a range of terpenoid compounds possess lice-repellent activity. Rosemary and Eucalyptus essential oils have similarly been demonstrated to possess repellent activity towards clothing lice (see Mumcuoglu *et al* (1996) *Entomologia Experimentalis et Applicata* 78: 309-314).

Ectoparasites of livestock, such as sheep, can detrimentally affect productivity of milk and meat, and the quality of wool and leather. Additionally, the welfare of animals infested with parasites can be seriously affected (see Bates (1999) in: Martin and Aitken (editors), *Diseases of Sheep* 3rd Ed., Chapter 45, Blackwell Science). Conventional treatment and control regimes are expensive to implement, and can be associated with health risks to the farmer.

Sheep scab results from infestation by *Psoroptes ovis* (sheep scab mite), and is usually treated by plunge dipping in washes containing organophosphates (such as diazinon or propetamphos) or synthetic pyrethroids (flumethrin or cypermethrin). Organophosphate dips have the advantage that a single immersion is

sufficient for treatment (see Bates (1999) in: Martin and Aitken (editors), *Diseases of Sheep* 3rd Ed., Chapter 46, Blackwell Science), but have been linked to health concerns for farmers (e.g. see Rees (1996). *Occupational and Environmental Medicine* 53: 258-263), whereas pyrethroid dips need to be used twice at 14 day intervals for effective treatment. *P. ovis* is usually restricted to sheep, although Psoroptic mange in cattle has been reported, and is a major problem in mainland Europe and the United States of America. The ear mite, *P. cuniculi*, is a close relative of *P. ovis*, and is primarily associated with rabbit populations, although it has also been isolated from sheep, goats and horses.

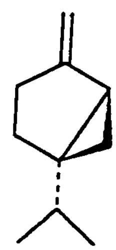
Chewing lice (*Bovicula ovis*) are another major ectoparasite of sheep, which can severely affect the quality of fleeces and hides. Treatment of lice is by similar means to the scab mite, by dipping in solutions containing organophosphates or synthetic pyrethroids. Pour-on treatments based on synthetic pyrethroids have been developed, but in Australia, resistance developed to these products within a few years of their release on to the market (see Bates (1999) in: Martin and Aitken (editors), *Diseases of Sheep* 3rd Ed., Chapter 45, Blackwell Science). The lipophilic nature of the components of the compositions described in the present invention make them ideal for development as pour-on treatments, for which lipophilicity is a key feature.

Sage contains a rich variety of chemicals, many of them terpenoids. They include amorphene, aromadendrene, borneol, bornyl acetate, cadinene, camphene, camphor, β-caryophyllene, caryophyllene oxide, 1,8-cineole (eucalyptol), copaene, cubebene, *p*-cymene, geraniol, germacrene D, gurjunene, α-humulene,

limonene, linalool, linalyl acetate, manool, myrcene, ocimene, palustrol, phellandrene, α-pinene, β-pinene, sabinene, sabinyl acetate, spathulenol, terpenyl acetate, terpinen-4-ol, terpinene, terpineol, terpinolene, α-thujone, β-thujone, viridifloral. Additional components of sage extracts could include (1R,5R)-epoxysalvial-4(14)-en-1-one, (2R,5E)-epoxycaryophyll-5-en-12-al, (2R,5E)-epoxycaryophyll-5-ene, (2S,5E)-epoxycaryophyll-5-en-12-al, 3-octanol, acetic-acid-ester, α-bisabolol, α-terpineol, α-terpinyl-acetate, arachidic acid, benzaldehyde, β-gurjuene, β-myrcene, β-sitosterol, butyric acid, caprylic acid, cerotinic acid, cis-3-hexen-1-ol, cis-allo-ocimene, cis-beta-ocimene, cis-linalol-oxide, citral, citronellol, cuminaldehyde, delta-3-carene, furfurol, gamma-terpinene, geranial, geraniol, geranyl acetate, isospathulenol, lignoceric acid, linoleic acid, linolenic acid, n-nonanol, n-pentanol, neral, nerol, nerolidol, neryl acetate, oleanolic-acid, oleic-acid, palmitic acid, phellandrene, propionic acid-ester, rosmarinic acid, salvia-4(14)-en-1-one, sclareol, stearic acid, terpinen-4-ol, trans-allo-ocimene, trans-β-ocimene, trans-β-terpineol, trans-linalool oxide, ursolic acid, valeric acid-ester, 5-hydroxy-6,7,4'-trimethoxyflavone, 6,8-di-C-glucosylapigenin, 6-hydroxylutein-6,3'-dimethylether, 6-methoxy apigenin-7-glucoside, 6-methoxy apigenin-7-glucuronide, 6-methoxy luteolin-7-glucoside, 6-methoxy luteolin-7-glucuronide, apigenin-7-glucoside,apigenin-7-glucuronide, betulinic-acid, carnesol, chrysoeriol-7-glucuronide, luteolin-7-glucoside, luteolin-7-glucuronide, luteolin-7-glucuronide-3'-glucoside, luteolin diglucoside, picrosalvin, salvigenin, (E)-nerolidol, 2,6-dimethyl-10-(*p*-tolyl)-undeca-2,6-diene, 2-octanol, 3-octanol, allo-aromadendrene, α-amorphene, α-copaene, α-cubebene, α-gurjunene, α-humulene, α-muurolene, α-phellandrene, α-selinene, β-cyclocitral,

cadina-1,4-diene, cis-α-bisabolene, δ-cadinene, γ-muurolene, isoamyl-acetate, isocaryophyllene, isopinocamphone, methyl perillate, myrtenol, myrtenyl acetate, perillaldehyde, perillyl acetate, perillyl alcohol, perillyl-butyrate, t-cadinol, trans-α-bergamotene, trans-calamenene and viridiflorene.

Sage essential oil samples which are most effective in killing parasites contain high concentrations of sabinene (up to 25%). Sabinene is a bicyclic monoterpenoid, of the formula $C_{10}H_{16}$, whose formula can be represented:



The parasite-killing activity of sabinene described in the present invention is previously unreported, although this compound has been shown to possess other biological activities. For example, US patent 5,635,184 to Camano describes potent antibacterial activity of the essential oil of *Schinus molle*, which contains significant amounts of sabinene. A composition based on volatile monoterpene compounds, one of which could include sabinene, has been described as possessing activity against house- and leopard mites (see Arakawa Chem Ind Ltd (1992) Japanese Patent 0413914A).

It is also desirable for these oils to have low concentrations of thujone and sabinyl acetate, which have been linked to mammalian toxicity (see Tisserand

(1995) *Essential Oil Safety- A Guide for Health Care Professionals;* Fournier *et al.* (1993) *Plant Medica* 59: 96-97). Spanish sage is particularly desirable in this regard, due to its low thujone content, and is classified as safe to use, being unlikely to cause toxicity, irritation or sensitisation. Despite the presence of thujone in other Sage essential oils, these have been determined to be of lower toxicity than expected on the basis of their thujone content alone, and are unlikely to cause irritation or sensitisation (see Tisserand (1990) *Essential Oil Safety Data Manual*). The same source also indicates that, for dermal application, essential oils of similar potential toxicity as thujone-rich sage oil could be applied twice weekly at concentrations between 1-2 and 3-5 % by volume. These values are all above the final concentration of sage oil in an aqueous-alcoholic carrier, as described in the present invention.

It is therefore an object of the present invention to provide treatments for parasitic infestations which are pleasant to use, effective, of low mammalian toxicity, and are unlikely to lead to the development of side-effects or adverse reactions.

Summary of invention

The ability of sabinene-containing essential oils, and in particular those from plants of the genus *Salvia* (sage), to kill insects, and in particular parasitic lice and mites is described. Essential oils, and in particular the oils from *Salvia lavandulifolia* and *S. officinalis* "petite feuille Banon", typically containing high concentrations of sabinene, and low concentrations of thujone, immobilise both head- and clothing lice almost immediately on contact, and

remain highly active when diluted down to 0.5 % (w/v) in an aqueous-alcohol carrier which is suitable for topical application to the head or skin. The same preparations are also effective treatments for sheep parasites, including *Psoroptes* (mite) and *Bovicula* (louse) species.

A number of terpenoid components of these essential oils, including sabinene, limonene, β-caryophyllene, myrcene and terpinen-4-ol are also described as possessing activity against the same parasites. It is further demonstrated that a number of terpenoid components of the sage oil synergistically interact, and kill ectoparasites at lower doses than when applied individually in an *in vitro* model.

Further embodiments of the invention are described in the accompanying claims.

Detailed description of invention

The present invention is based on the discovery that essential oils can effectively kill insects, especially parasitic insects such as ectoparasites. Certain oils, including those from *Salvia* species and other plant species containing relatively high concentrations of sabinene have been found, surprisingly, to be most effective. Species of *Salvia* suitable for use in the present invention include *Salvia aethiopis, Salvia amissa, Salvia apiana, Salvia argentea, Salvia arizonica, Salvia azurea, Salvia ballotiflora, Salvia blodgettii, Salvia brandegei, Salvia carduacea, Salvia carnosa, Salvia chapmanii, Salvia chia, Salvia clevelandii, Salvia coccinea, Salvia columbariae, Salvia davidsonii, Salvia divinorum, Salvia dolichantha, Salvia dorrii, Salvia*

earlei, Salvia engelmannii, Salvia eremostachya, Salvia farinacea, Salvia funerea, Salvia glutinosa, Salvia grahamii, Salvia greatae, Salvia greggii, Salvia henryi, Salvia hispanica, Salvia lancifolia, Salvia lemmonii, Salvia leptophylla, Salvia leucophylla, Salvia longistyla, Salvia lycioides, Salvia lyrata, Salvia mellifera, Salvia micrantha, Salvia microphylla, Salvia misella, Salvia mohavensis, Salvia munzii, Salvia nemorosa, Salvia nutans, Salvia occidentalis, Salvia officinalis, Salvia pachyphylla, Salvia parryi, Salvia penstemonoides, Salvia pinguifolia, Salvia pitcheri, Salvia potus, Salvia pratensis, Salvia privoides, Salvia ramosissima, Salvia reflexa, Salvia regla, Salvia riparia, Salvia roemeriana, Salvia sclarea, Salvia serotina, Salvia sonomensis, Salvia spathacea, Salvia splendens, Salvia subincisa, Salvia summa, Salvia texana, Salvia thomasiana, Salvia tiliifolia, Salvia urticifolia, Salvia vaseyi, Salvia verbenacea, Salvia verticillata, Salvia vinacea, Salvia virgata, Salvia X *bernardina, Salvia* X *palmeri, Salvia* X *superba* and *Salvia* X *sylvestris.* Particularly preferred are *Salvia lavandulifolia* or *S. officinalis.* Other species suitable for use in the present invention include *Artemesia dracumculus* (Tarragon), *Citrus limon* (lemon), *Juniperus communis* (Juniper), *Laurus nobilis* (Bay), *Myristica fragrans* (Nutmeg), *Origanum Vulgare* (Oregano), *Piper cubeba* (Cubebs) and *Aloysia gratissima* (Whitebrush). The oils can be formulated into both aqueous and non-aqueous formulations which remain active against lice. In a mixed alcoholic-aqueous carrier, concentrations of oil required to kill human lice are comparable to those of the (organophosphate-based) active components in commercial head lice treatments.

Sabinene content of essential oil samples can be elevated by mixing with ethanol, and partitioning the resulting solution with known volumes of water. The most effective ratio of solvents for these experiments is 5 volumes of ethanol:water (3:2) per volume of essential oil, although a range of ratios, from 1:1:1 to 6:4:1 (ethanol:water:essential oil) are also effective. When the partitioning is repeated, the resulting oil shows an increased content of sabinene, up to 25 % by weight.

Previously, the lice-repellency activity of *Salvia sclarea* essential oil and terpenoid components characteristic of this, and related oils, has been described (see Eini *et al.* 1995 US Patent 5,411,992). The lice- and mite-killing activity of sage essential oils, and their chemical components is previously unreported.

Activity of essential oils and terpenoid components of essential oils was tested using an *in vitro* model system. Test solutions were prepared by accurately dissolving and mixing essential oils or terpenoid components in a range of carrier solutions, including water, isopropanol (propan-2-ol; IPA), water-IPA mixtures in the range 10-90 % by volume IPA and inactive vegetable oils, such as grape seed oil. Clothing lice (*Pediculus humanus humanus*), head lice (*Pediculus humanus capitis*) or sheep biting lice (*Bovicula ovis*) were then transferred to small volumes (typically 1-2 ml) of these test solutions, in glass containers, and were left in contact with the solutions for 10 minutes. During this time the solutions were occasionally shaken gently to ensure adequate contact between the lice and the test solution. After 10 minutes, the lice were removed, and placed on filter paper to remove excess treatment.

Exposure of lice to test substances in this way (treatments or controls) resulted in the lice being temporarily immobilised. The lice were then monitored for signs of physical activity for a period of up to 90 minutes. Lice exposed to non-active test substances or controls typically regained activity within 5 minutes of their removal from the test solution; those which failed to regain any activity within a post-treatment period of 30-90 minutes were classified as dead.

Due to their smaller size, parasitic mites (*Psoroptes ovis* or *P. cuniculi*) were treated by adding droplets of test solution to the parasites. The time taken for the mites to stop moving in the test solution was taken as an indication of the toxicity of the test substance. In all other respects, the experiments were performed similarly to those used to study parasitic lice.

Each experiment was performed at a range of concentrations of the test substance, to determine the dose-response of these materials. Activity of the treatments was expressed as an LD_{50}, which was the concentration of essential oil or terpenoid compound sufficient to kill half of the parasites exposed to that treatment.

Example 1a
Activity of sabinene-containing essential oils against human parasitic lice

At high concentrations, sage essential oil led to gross morphological disruption of the human lice, characterised by abdominal swelling, and the development of an intense red coloration in the body and limbs. When dissolved in an inert (no activity against lice) carrier oil, the LD_{50} for sage essential oil containing as little as 5 % by weight sabinene was in the concentration range of 250-300 $mgml^{-1}$ (Table 1, FIG. 1). In a carrier consisting of IPA (20 % v/v) in water, however, the LD_{50} of the same oil was much lower, at between 3-4 $mgml^{-1}$ (Table 2a, FIG. 2). As head lice (*Pediculus humanus capitis*) were slightly more sensitive to the sage oil formulations than clothing lice (*P. humanus humanus*) (Tables 2a and 2b), subsequent experiments were performed using clothing lice as the model system, as concentrations of oils effective against these lice would also be effective against head lice.

Table 1: Effect of concentration of sage oil in vegetable oil carrier on clothing lice recovery (%)

Concentration ($mgml^{-1}$)	Time post-treatment (minutes) 0	2	5	10	15	30
0	0	60	80	80	100	100
50	0	40	60	60	60	80
100	0	40	60	60	60	80
150	0	60	60	60	60	60
200	0	40	60	60	60	60
250	0	20	40	40	60	60
300	0	0	0	0	20	20

Table 2a: Effect of concentration of sage oil in 20% (v/v) IPA-water on clothing lice recovery (%)

Concentration	Time post-treatment (minutes)					
(mg ml^{-1})	0	2	5	10	15	30
0	0	0	100	100	100	100
1	0	0	100	100	100	100
2	0	33	100	100	100	100
3	0	33	33	33	33	100
4	0	0	33	33	33	33
5	0	0	0	0	0	0
10	0	0	0	0	0	0
15	0	0	0	0	0	0
20	0	0	0	0	0	0

Table 2b: Effect of concentration of sage oil in 20% (v/v) IPA-water on head lice recovery (%)

Concentration	Time post-treatment (min)				
($mgml^{-1}$)	0	2	5	10	30
Control	0	25	50	100	100
1	0	33	67	67	33
2	0	29	43	57	29
3	0	25	50	50	50
4	0	0	0	0	0
5	0	0	0	0	0
10	0	0	0	0	0

Addition of isopropanol (IPA), in the range 0-20% (v/v) led to increased activity of the sage oil components, with the IPA itself having no direct effect of the lice over this concentration range. At concentrations of 20% IPA (v/v) in inert (grapeseed) oil, 66-100% of lice treated in this manner recovered activity within 10 minutes. With the addition of a sub-LD_{50} dose (150 $mgml^{-1}$) of sage oil to the grapeseed oil, however, a clear dose response was apparent with increasing concentrations of IPA (Table 3).

Table 3: Effect of concentration of IPA in vegetable oil containing 150 $mgml^{-1}$ sage essential oil on clothing lice recovery (%)

IPA concentration (% v/v)	Time post-treatment (minutes)				
	0	5	10	15	30
5	0	57	71	71	71
10	0	33	50	50	50
15	0	0	0	0	13
20	0	0	0	0	0

Four separate sage oil samples, dissolved in 20% IPA in water were tested for activity against lice at 5 $mgml^{-1}$. Table 4 clearly shows that lice killing activity of these oils was proportionally related to their sabinene content. Data from gas chromatographic analysis of the most active oil sample (sample 1, *S. officinalis* "petite feuille Banon"), is given in table 5.

Table 4: Effect of four sage oils samples on clothing lice recovery (%)

Sage oil sample	Sabinene content weight (%)	Time post-treatment		
		0 min	10 min	90 min
1	20.3	0	0	0
2	4.8	0	0	20
3	0.01	0	40	50
4	0.6	0	20	40

Other oils containing relatively large amounts of sabinene were tested in a similar manner. At a concentrations of between 2 and 10 $mgml^{-1}$ in the IPA-water carrier, the essential oils from cubebs (*Piper cubeba*) and lemon (*Citrus limon*) were most active, killing 100% and 66% of clothing lice, as assessed 60

minutes after treatment. In the vegetable oil carrier system (at concentrations of 300 mgml^{-1}), nutmeg (*Myristica fragrans*), lemon (*Citrus limon*) and tarragon (*Artemisia dracunculus*) were also all active against at least 66% of the clothing lice treated. Concentrations of *Salvia lavandulifolia* essential oil of as little as 4 mgml^{-1} in 20% by volume IPA-water were sufficient to kill all treated lice in some experiments, compared to 6 mgml^{-1} for the essential oil of tea tree (*Melaleuca alternifolia*), which is commonly used as an "alternative" treatment for head lice, indicating sage oil is more potent than tea-tree oil against these lice.

Table 5: Identified chemical components of the essential oil of *Salvia officinalis* "petite feuille Banon"

Retention time (min)	Area	Name
3.460	1248525	α-pinene
3.806	7163	camphene
4.453	3102985	sabinene
4.993	1835	myrcene
5.781	305	limonene
6.309	4182618	1,8-cineole
6.608	45281	*cis*-β-ocimene
6.987	345763	*trans*-β-ocimene
7.978	70112	*p*-cymene
12.887	531617	α-thujone
13.648	156916	β-thujone
17.021	2136359	camphor
18.891	109529	linalool
21.110	1086325	β-caryophyllene
21.339	6682	terpinen-4-ol
24.735	1124187	α-humulene
26.546	283123	α-terpineol
26.641	879488	borneol

Example 1b

Activity of sabinene-containing essential oils against parasites of non-human species

When dissolved in an inert carrier oil, the LD_{50} for sage essential oil containing as little as 5 % by weight sabinene against ear mites (*Psoroptes cuniculi*) was in the concentration range of 50-100 $mgml^{-1}$ (FIG. 4). In a carrier consisting of IPA (20 % v/v) in water, however, the LD_{50} of the same oil was lower, at between 4-5 $mgml^{-1}$ (FIG. 5). Against biting lice (*Bovicula ovis*), sage oil in an inert oil carrier at 300 $mgml^{-1}$ or in 20% by volume IPA-water at 5 $mgml^{-1}$ was highly effective at killing lice after an exposure time of 10 minutes.

Example 2a

Activity of terpenoid essential oil components against human parasitic lice.

A number of terpenoid components of the essential oil of sage are commercially available. A range of these compounds were tested for relative activity by dissolving/mixing the compounds at equimolar concentrations in inert vegetable carrier oil. For the comparative studies, all components were prepared to a concentration of 0.97M (approximately 125-200 $mgml^{-1}$, depending on molecular weight). To avoid only partial solubility in predominantly aqueous carrier media, vegetable oil was selected as the carrier was these studies; all compounds were fully soluble in this medium.

These experiments demonstrated that there was great variation in the activity of the essential oil components against clothing lice, ranging from complete activity (sabinene) through to no detectable activity (1,8-cineole). The other components showed intermediate

activity, with limonene and caryophyllene the most active components excluding sabinene (FIG. 3, Table 6).

Table 6: Effect of sage oil components on recovery (%) of clothing lice
(all compounds at equimolar concentrations in carrier oil)

Compound	Time post-treatment (min)					
	0	2	5	10	15	20
borneol	0	0	50	50	50	50
camphene	0	50	50	50	50	50
camphor	0	0	50	50	50	50
β-caryophyllene	0	0	0	0	25	50
1,8-cineole	0	50	100	100	100	100
p-cymene	0	0	50	50	50	50
limonene	0	0	0	25	25	25
linalool	0	0	50	100	100	100
myrcene	0	0	0	50	50	50
α-pinene	0	50	50	50	50	50
sabinene	0	0	0	0	0	0
terpinen-4-ol	0	0	0	50	50	50
terpineol	0	50	50	50	50	50
Carrier oil	0	0	100	100	100	100

Example 2b

Activity of terpenoid essential oil components against parasites of non-human species.

Against *Psoroptes cuniculi*, as with the human parasitic lice, there was great variation in the activity of the essential oil components, ranging from complete activity (linalool) through to no detectable activity (caryophyllene). The other components showed intermediate activity, with both limonene and sabinene displaying complete activity within 5 minutes of application to the mites (FIG. 6).

Example 3

Activity of sage oil compared to conventional

treatments.

Initial studies indicate that the use of sage oil at 5 $mgml^{-1}$ in 20% by volume IPA-water is more effective in killing lice than synthetic insecticides used in commercial treatments on a weight-weight basis in the same solvent system. For example, clothing lice treated with 5 $mgml^{-1}$ sage oil in 20% by volume IPA-water showed no recovery of activity, whereas lice treated similarly with 5 $mgml^{-1}$ malathion or permethrin in 20% by volume IPA-water showed signs of recovery within 1 hour. The legs and antennae of approximately 80% of malathion-treated lice showed twitching motion 40 minutes after treatment, whilst lice exposed to permethrin were able to move (albeit slowly) within 45 minutes of treatment. In both these cases, all the treated lice were able to undertake locomotion within 48 hours of treatment. No such activity was observed in lice treated with 5 $mgml^{-1}$ sage oil.

Example 4

Interaction between isolated monoterpene essential oil components

To test whether isolated terpenoid components of sage essential oils were able to interact with each other, and kill lice at concentrations below their LD_{50} values when tested in isolation, sub-lethal concentrations of the compounds listed in table 4 were prepared in carrier oil. In practice, the concentrations tested were as follows: myrcene (0.24 M), β-caryophyllene (0.24 M), *p*-cymene (0.25 M), terpinen-4-ol (0.32 M), linalool (0.32 M), sabinene (0.37 M), α-terpineol (0.43 M), 1,8-cineole (0.43 M), limonene (0.48 M) and α-pinene (0.73 M). At these concentrations, clothing lice rapidly recovered activity, typically within 5 minutes of treatment. Combinations of sabinene with limonene, sabinene with α-

terpineol and limonene with terpinen-4-ol and all demonstrated increased activity against lice, compared to the isolated compounds. In these combinations of compounds, the lice failed to recover activity within 5 minutes, whereas the lice exposed to the isolated compounds at sub-lethal concentrations were fully active by this time.

Against ear mites (*Psoroptes cuniculi*), combinations of sabinene with α-terpineol, linalool with α-terpineol and linalool with *p*-cymene were all more active than their monoterpene components applied to the mites in isolation.

Example 5

Other natural compounds active against lice.

In addition to sabinene-containing essential oils, extracts from the bulbs of plants of the genus *Narcissus* were also determined to display potent activity against lice. Galanthamine, an alkaloid found in such extracts, was determined to be particularly active in this regard, and was completely effective against lice at concentrations of 5 $mgml^{-1}$ in 20 % IPA (v/v) in water. The lice-killing activity of galanthamine was possibly due to its potent ability to inhibit acetylcholinesterase, which was determined using spectrophotometric methods.

CLAIMS:

1. A composition comprising the terpenoid sabinene at a concentration of 4 to 25% by weight for use in the treatment of a human or animal having a parasitic insect infestation.

2. A composition for use as claimed in claim 1 which further comprises limonene or α-terpineol or a mixture thereof.

3. A composition for use as claimed in claim 1 or claim 2 which comprises the essential oil or an extract of a sabinene-containing plant.

4. A composition for use as claimed in claim 3 wherein the plant is selected from the genera *Salvia, Artemesia, Citrus, Juniperus, Laurus, Myristica, Origanum, Piper* or *Aloysia*.

5. A composition for use as claimed in claim 4 wherein said plant is *Salvia lavandulifolia* or *Salvia officinalis*.

6. A composition comprising the essential oil or an extract of a plant selected from the genera *Salvia, Artemisia, Citrus, Juniperus, Laurus, Myristica, Origanum, Piper* or *Aloysia* for use in the treatment of a human or animal having a parasitic insect infestation.

7. A composition for use as claimed in claim 6 comprising the essential oil or an extract of *Salvia lavandulifolia* or *Salvia officinalis*.

8. A composition for use as claimed in claim 6 or

sabinene.

9. A composition for use as claimed in any one of the preceding claims which is formulated for topical application to said human or animal.

10. A composition for use as claimed in any one of claims 3 to 10 wherein said essential oil or extract or any insecticidally active fraction thereof is dissolved in an oil-based medium or a water/alcohol based medium.

11. A composition for use as claimed in claim 9 formulated as a hair conditioner, mousse or shampoo.

12. A composition for use as claimed in claim 9 formulated as a dip or pour-on treatment.

13. A composition as claimed in claim 10 wherein said water/alcohol based medium comprises from 10 to 90% alcohol by volume, preferably about 20%.

14. A composition as claimed in claim 13 wherein said alcohol is isopropyl alcohol.

15. A composition comprising the alkaloid galanthamine for use in the treatment of a human or animal having a parasitic insect infestation.

16. A composition for use as claimed in claim 15 wherein said composition comprises an extract of a plant of the genus *Narcissus*.

17. A composition for use as claimed in claim 15 or claim 16 wherein said galanthamine or extract is dissolved in an oil-based medium, a water/alcohol based medium or is formulated as a hair conditioner or shampoo or as a dip or pour-on treatment.

or as a dip or pour-on treatment.

18. A composition for use as claimed in any one of the preceding claims wherein said parasitic insects are selected from lice, lice eggs, mites, fleas or parasites associated with blowfly strike.

19. A composition for use as claimed in claim 18 wherein said parasitic insects are selected from head lice (*Pediculus humanus capitis, syn. P. capitis*), clothing lice (*Pediculus humanus humanus syn. P. corporis*), pubic lice (*Pthirius pubis*), biting lice (*Bovicula ovis*), scab mite (*Psoroptes ovis*), ear mite (*Psoroptes cuniculi*), dust mites (primarily of the genus *Dermatophagoides*, pig mites, cat fleas (*Ctenocephalalides felis*), dog fleas (*C. canis*), horse fleas and *Lucilia* or *Chrysomya* species.

20. Use of the terpenoid sabinene in the manufacture of a composition for the treatment of a human or animal having a parasitic insect infestation.

21. The use as claimed in claim 20 wherein said composition comprises sabinene at a concentration of 4 to 25% by weight.

22. The use as claimed in claim 20 or claim 21 wherein said composition is characterized by a feature as defined in any one of claims 2 to 5 and 9 to 14.

23. Use of the essential oil or an extract of a plant of the genera selected from *Salvia, Artemisia, Citrus, Juniperus, Laurus, Myristica, Origanum, Piper* or *Aloysia* in the manufacture of a composition for the treatment of a human or animal having a parasitic insect infestation.

24. The use as claimed in claim 23 wherein said composition is characterized by a feature as defined in any one of claims 7 to 14.

25. Use of the alkaloid galanthamine in the manufacture of a composition for the treatment of a human or animal having parasitic insect infestation.

26. The use as claimed in claim 25 wherein said composition is characterized by a feature as defined in claim 16 or claim 17.

27. The use as claimed in any one of claims 20 to 26 wherein said poarasitic insects are selected from lice, lice eggs, mites, fleas or parasites associated with blowfly strike.

28. The use as claimed in claim 27 wherein said parasitic insects are selected from head lice (*Pediculus humanus capitis, syn. P. capitis*), clothing lice (*Pediculus humanus humanus syn. P. corporis*), pubic lice (*Pthirius pubis*), biting lice (*Bovicula ovis*), scab mite (*Psoroptes ovis*), ear mite (*Psoroptes cuniculi*), dust mites (primarily of the genus *Dermatophagoides*, pig mites, cat fleas (*Ctenocephalalides felis*), dog fleas (*C. canis*), horse fleas and *Lucilia* or *Chrysomya* species.

29. A method suitable for treating furnishing or clothing to kill parasitic insects which comprises exposing said furnishings or clothing to an insecticidal composition comprising the essential oil or an extract of a plant selected from the genera *Salvia, Artemisia, Citrus, Juniperus, Laurus, Myristica, Origanum, Piper* or *Aloysia*.

30. A method as claimed in claim 29 wherein said

composition is characterised by a feature as defined in any one of claims 7, 8, 10, 13 or 14.

31. A method as claimed in claim 30 wherein said composition comprises sabinene at a concentration of 4 to 25% by weight.

32. A method as claimed in claim 30 or claim 31 wherein said composition comprises sabinene and limonene or sabinene and α-terpineol or sabinene, limonene and α-terpineol.

33. A method suitable for treating furnishings or clothing to kill parasitic insects which comprises exposing said furnishings or clothing to an insecticidal composition comprising the alkaloid galanthamine.

34. A method as claimed in claim 33 wherein said composition includes an extract of a plant of the genus *Narcissus*.

35. A method as claimed in any of claims 29 to 35 wherein said parasitic insects are from head lice (*Pediculus humanus capitis, syn. P. capitis*), clothing lice (*Pediculus humanus humanus syn. P. corporis*), pubic lice (*Pthirius pubis*), biting lice (*Bovicula ovis*), scab mite (*Psoroptes ovis*), ear mite (*Psoroptes cuniculi*), dust mites (primarily of the genus *Dermatophagoides*, pig mites, cat fleas (*Ctenocephalalides felis*), dog fleas (*C. canis*), horse fleas and *Lucilia* or *Chrysomya* species.

36. A method suitable for treating plants to kill parasitic insects which comprises applying a composition comprising the terpenoid sabinene to said plants.

37. A method as claimed in claim 36 wherein said composition comprises sabinene at a concentration of 4

to 25% by weight.

38. A method as claimed in claim 36 or claim 37 wherein said composition is further characterized by a feature as defined in any one of claims 3 to 5 or 10 to 14.

39. A method for treating plants to kill parasitic insects which comprises applying to said plants a composition comprising the essential oil or an extract of a plant selected from the genera *Salvia, Artemisia, Citrus, Juniperus, Laurus, Myristica, Origanum, Piper* or *Aloysia*.

40. A method as claimed in claim 39 wherein said composition is further characterized by a feature as defined in any one of claims 7, 8, 10, 13 or 14.

41. A method of treating plants to kill parasitic insects which comprises applying to said plants a composition comprising the alkaloid galanthamine.

42. A method as claimed in claim 41 wherein said composition comprises an extract of a plant of the genus *Narcissus*.

43. A method as claimed in any one of claims 36 to 42 wherein said parasitic insects are selected from the genera *Aphis, Chilo, Dysdercus, Megoura, Musca, Pieris, Nilaparvata, Nephotettix* or *Tetranychus*.

FIGURE 1

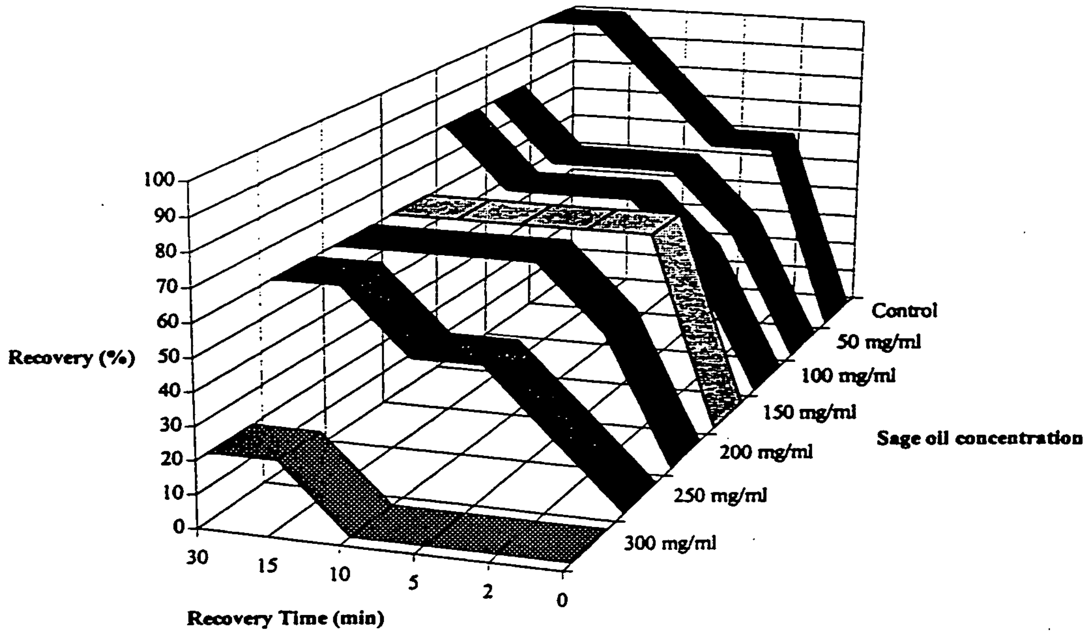


FIGURE 2

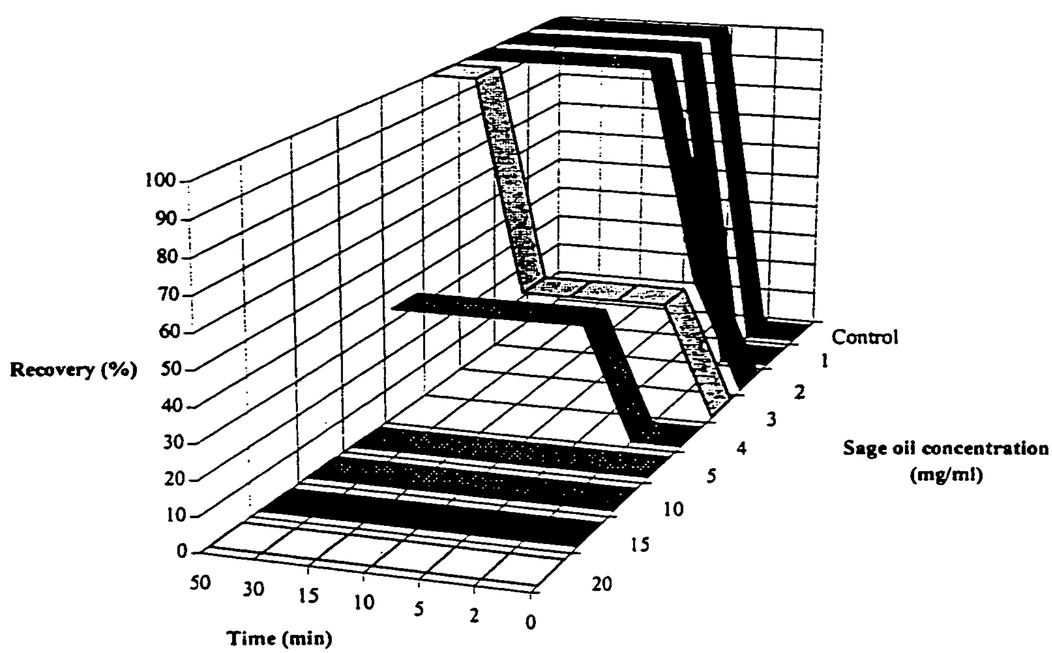


FIGURE 3

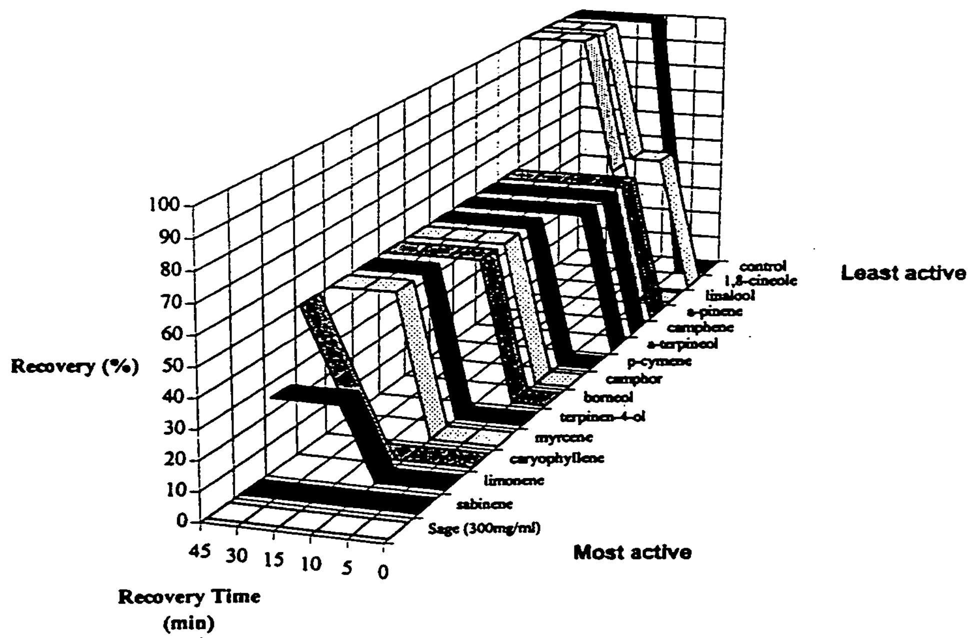


FIGURE 4

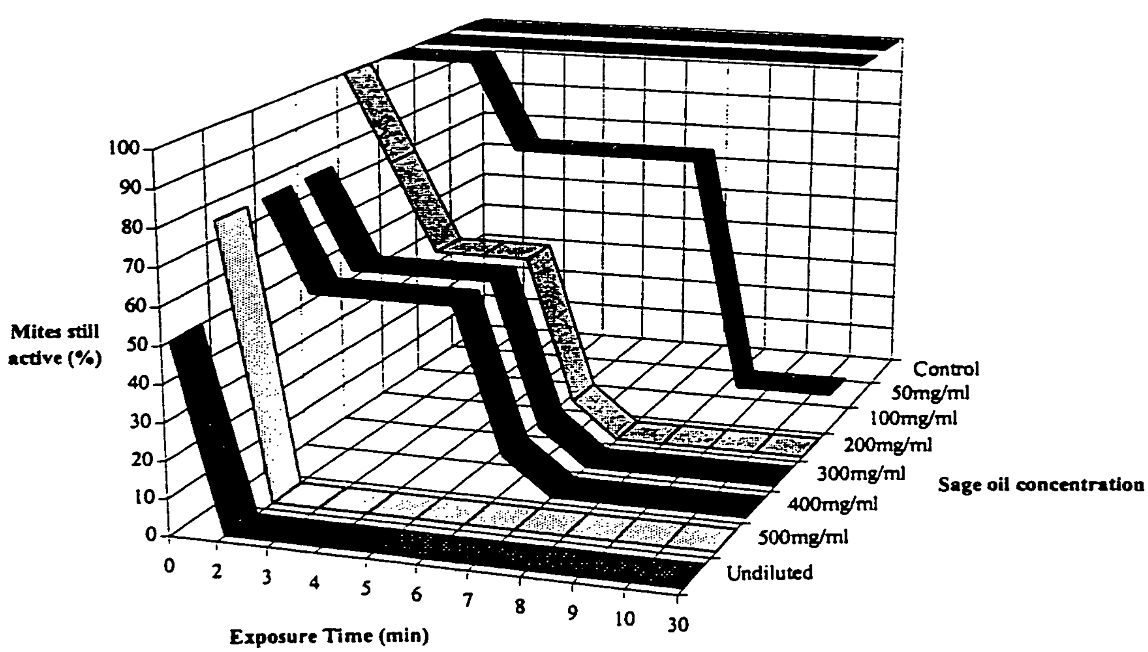


FIGURE 5

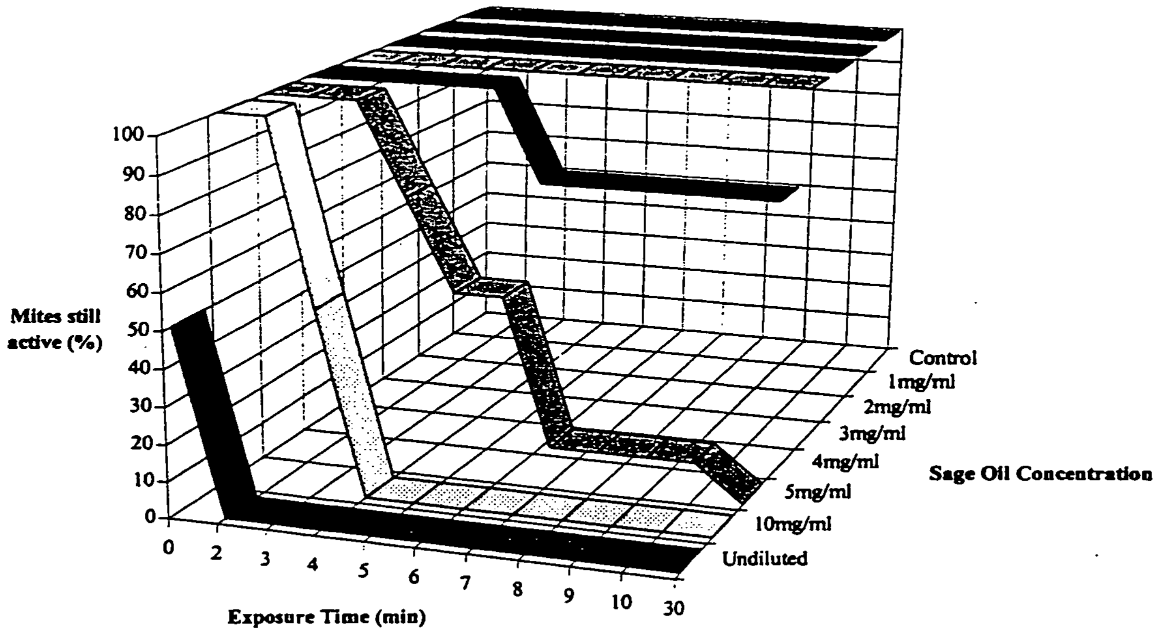


FIGURE 6